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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/698,343	10/31/2003	Manfred R. Koller	ONCOSIS.005A	3787
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KNOBBE MARTENS OLSON & BEAR LLP			FERNANDEZ, SUSAN EMILY	
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IRVINE, CA 92614			1651	

DATE MAILED: 01/27/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

10/698,343

Applicant(s)

KOLLER ET AL.

Examiner

Susan E. Fernandez

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 14 November 2005.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-18,21-34 and 45-56 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-18,21-34 and 45-56 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|-----------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input checked="" type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. <u>20050901</u> . |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| Paper No(s)/Mail Date _____. | 6) <input type="checkbox"/> Other: _____. |

DETAILED ACTION

The amendment filed November 14, 2005, has been received and entered. The text of those sections of Title 35, U.S. Code, not included in this action can be found in a prior office action.

Claims 1-18, 21-34, and 45-56 are pending and are examined on the merits.

Claim Rejections - 35 USC § 112

Claims 1-18, 21-34, and 45-56 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is rendered indefinite by the phrase "...without prior knowledge of the location of said one or more cells..." The phrase is confusing in that it is unclear how one would practice the process as claimed without knowing the location of one or more cells. To a certain degree, the position of any vessel containing the cells (hence the position of the cells) should be known to ensure that there even exists a probability that one or more cells is/are coincident with the path of the electromagnetic radiation. Moreover, there is clearly prior knowledge of the location of said one or more cells, since the claim steps clearly indicate the location of the cells (cells are located in the container). Thus, claims 1-18, 21-34, and 45-47 are rejected under 35 U.S.C. 112, second paragraph. To overcome this rejection, it is suggested that the phrase be substituted with the following: "...without prior knowledge of the location of said one or more cells **in said container...**"

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Claims 45, 48, and 56 are indefinite since it is unclear what is defined by the recitation “effective distance.” It is not clear what makes the distance “effective,” nor is it clear what is the desired effect. Applicant's arguments filed November 14, 2005, have been fully considered but they are not persuasive. Note that it is improper to read limitations into the claims from the specification. According to M.P.E.P. 2111.01, “While the claims of issued patents are interpreted in light of the specification, prosecution history, prior art and other claims, this is not the mode of claim interpretation to be applied during examination. During examination, the claims must be interpreted as broadly as their terms reasonably allow.” Though the applicant can be his or her own lexicographer, purported definition in paragraph [0073] does not define the term “effective distance” with reasonable clarity, deliberateness, and precision. Thus, claims 45 and 48-56 are rejected under 35 U.S.C. 112, second paragraph.

Claim 47 and 56 is rendered indefinite by the term “substantially.” It is not clear what would constitute a “substantially stationary position.” Applicant's arguments have been fully considered but they are not persuasive. Note that it is improper to read limitations into the claims from the specification. According to M.P.E.P. 2111.01, “While the claims of issued patents are interpreted in light of the specification, prosecution history, prior art and other claims, this is not the mode of claim interpretation to be applied during examination. During examination, the claims must be interpreted as broadly as their terms reasonably allow.” Though the applicant can be his or her own lexicographer, the purported definition in paragraph [0073] does not define the phrase “substantially stationary position” with reasonable clarity, deliberateness, and precision.

Claims 48 and 56 are rendered indefinite by the phrase "...without prior knowledge of the specific three-dimensional location of said one or more cells..." The phrase is confusing in that it is unclear how one would practice the process as claimed without knowing the location of one or more cells. To a certain degree, the position of the solid surface (hence the position of the cells) should be known to ensure that there even exists a probability that one or more cells is/are coincident with the path of the electromagnetic radiation. Applicant's arguments have been fully considered but they are not persuasive. One of ordinary skill in the art clearly must know that the one or more cells are located within an effective distance from a solid surface, and anywhere within the area of said solid surface, and this knowledge can be considered knowledge of the "specific three-dimensional location of said one or more cells." Thus, claims 48-56 are rejected under 35 U.S.C. 112, second paragraph.

The phrase "modified nucleic acid," renders claims 16 and 18 indefinite. It is not clear what modifications are appropriate for a nucleic acid to be considered "modified." Applicant's arguments have been fully considered but they are not persuasive. The specification offers only one example of a "modified nucleic acid" and does not clearly define each and every compound that is considered a "modified nucleic acid." Thus, the metes and bounds of the term are unclear, as the phrase could encompass any and all compounds, depending on the extent a nucleic acid is "modified."

Claim Rejections - 35 USC § 102

Claims 1, 6-9, 14, 17, 18, 23, 25-29, 32, 45-48, 51, 55, and 56 are rejected under 35 U.S.C. 102(b) as being anticipated by Kasuya et al. (US 5,013,660).

Kasuya et al. discloses apparatuses and a method for transiently permeabilizing cells by the application of a laser beam, where the source may be a pulsed laser or a continuous wave laser. In particular, see claims 1-4, 6, and 7. Figure 4 offers one embodiment of an appropriate apparatus for membrane permeabilization (column 5, lines 7-66). The given apparatus comprises of a laser source and a sample holder containing the “cells floating in a solution” (column 5, line 29). Moreover, since the sample is contained in the sample holder held on a stage (14 on Figure 4), the cells are also considered to be in a substantially stationary position within a certain distance from a solid surface, which is either the top or bottom of the sample holder.

Prior to permeabilization, the only information obtained is the planar distribution of the cells without any specifics in terms of coordinates (column 5, lines 32-37). Therefore, there is knowledge of the specific two-dimensional location of the cell, but there is no prior knowledge of the specific three-dimensional location of the cells. Thus, this embodiment meets the requirement of directing the electromagnetic radiation “without prior knowledge of the specific three-dimensional location of said one or more cells” as recited in claims 48 and 56 under examination.

The laser beam may be directed “across a predetermined area of the suspension” (claim 4), and this would be accomplished with the various components of the apparatus (column 5, lines 10-48), or by moving the sample holder (column 5, lines 62-66). Laser beam pulses are delivered to the sample holder, thus to the solid surface of the top or bottom of the sample holder. Furthermore, the beam passes in a path pattern since the beam is swept across the solid surface in order to strike most or all of the “predetermined area of the suspension” or the “cell-floating area” (column 5, line 64). The beam pulse targets a “defined area” which could be

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considered either the entirety or a portion of the “cell-floating area”. Moreover, the “cell-floating area” can be considered the entire area of the bottom of the sample holder.

Example 1 offers specific parameter values, including laser beam pulse duration of 10 nanoseconds (column 4, line 32), and a pulse frequency of 10 Hz (column 4, line 34). Thus, the electromagnetic radiation is directed at the cells for a period of at most on the order of 10 nanoseconds, 100 nanoseconds, 1 microsecond, 10 microseconds, 100 microseconds, 1 millisecond, 10 milliseconds, 100 milliseconds, 1 second, 10 seconds, 100 seconds, or 1000 seconds. Moreover, the pulse frequency is at least 1 or 10 Hz. Additionally, Example 1 specifies that a sample of cells can be in a medium comprising DMEM added with 10% unborn calf's blood serum (column 4, lines 25-26), which is considered a non-isotonic aqueous medium.

Note that Example 1 (column 4, lines 15-42) is an embodiment of the Kasuya invention wherein there is no knowledge of the location of the cells (only that the cells are floating in the medium). There is no cell detection step, thus there is no prior knowledge of the location of the cells in the medium.

The method allows for the entry of foreign substances into the permeabilized cells, where the foreign substances are introduced by means of the liquid medium. The foreign substance may be “DNA, protein or any other biopolymer” (column 2, lines 19-20), thus it may be an organic molecule, a peptide, a protein, a nucleic acid, or a modified nucleic acid. Furthermore, after entry due to transient permeabilization, the cells return to their previous state within a few seconds (column 5, lines 57-61). Therefore, the transiently permeabilized membrane recovers to its previous state within a period of time consisting of at most about 3 seconds, 10 seconds, 30 seconds, 1 minute, 2 minutes, 3 minutes, 6 minutes, 10 minutes, 20 minutes, or 30 minutes.

Applicant's arguments have been fully considered but they are not persuasive. With respect to the claims requiring that there is no prior knowledge of the “specific three-dimension location of said one or more cells” (claims 48-56), it is respectfully pointed out that the embodiments of Kasuya et al. discussed in the previous office action (column 5), are drawn to the detection of the planar distribution of the cells, or in other words, the two-dimensional distribution of the cells. Thus, this particular embodiment referenced in the previous office action discloses all of the features of independent claims 48 and 56 under examination.

While Kasuya et al. teaches embodiments wherein a cell with exposed to electromagnetic radiation only after receiving knowledge of the location of the cell, it is also respectfully pointed out that Example 1 is one embodiment of the Kasuya invention wherein there is no detection of a cell's location (thus there is no knowledge of a cell's location in medium). Thus, Example 1 teaches all the features of instant independent claim 1, as amended.

Thus, the rejections of the claims over Kasuya et al. are appropriate and must be maintained.

Claim Rejections - 35 USC § 103

Claims 1-3, 6-14, 17, 18, 23, 25-34, 45-48, 51, and 53-56 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kasuya et al. in view of Koller et al. (US 2002/0076744).

As discussed above, Kasuya et al. anticipates claims 1, 6-9, 14, 17, 18, 23, 25-29, 32, 45-48, 51, 55, and 56. However, Kasuya et al. does not expressly disclose the laser beam energy density, rate of permeabilization, cell viability following transient permeabilization, certain substances recited in claim 18, the area of the “defined area”, or the path width of the laser beam.

Koller et al. discloses a method for transiently permeabilizing a target cell wherein the target cell is located in a population of substantially stationary cells, and then irradiated with a pulse of radiation (claim 1) from an energy beam, such as a laser (page 2, paragraph [0020]). The radiation may have energy densities as listed on page 9, paragraph [0092], thus the energy density is at most about 0.001, 0.002, 0.003, 0.006, 0.01, 0.02, 0.03, 0.06, 0.1, 0.2, 0.3, 0.6, 1, or 2 $\mu\text{J}/\mu\text{m}^2$. Koller et al. also discloses the permeabilization rate in terms of cells per minute (page 9, paragraph [0094]), where the minimum rate is about 83 cells per second, and no upper limit is given. Therefore, transient permeabilization is induced at a rate of at least 10, 30, 100, 300, 1000, 3000, 10,000, 30,000, 100,000, 300,000, 1,000,000, 3,000,000, 10,000,000, 30,000,000, 100,000,000, or 240,000,000 cells per second. Additionally, the lower limits of the probability of viability of irradiated cells are disclosed, where no upper limits are offered (page 8, paragraph [0084]). Koller et al. thus teaches a probability of viability of at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99%. Claims 23-25 recite areas of the frame comprising the cell population, which can be considered the “defined area”. At a minimum, the area is at least 0.5 cm^2 , thus the “defined area” has an area of at least 1, 3, 10, 30, 100, 200, 300, or 400 cm^2 . With respect to the path width of the energy beam, various diameters are listed on page 9, paragraph [0091], all of which are lower limits. Therefore, the path width is at least 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 300, 1×10^3 , 2×10^3 , 3×10^3 , 4×10^3 , 5×10^3 , 6×10^3 , 7×10^3 , 8×10^3 , 9×10^3 , or 1×10^4 micrometers. Finally, the foreign material that may enter the permeabilized cell include nucleic acids, polypeptides, polysaccharides, lipids, dextran (colloid), and small molecules. Since small molecules are included, ions and inorganic molecules must also be able to enter the cell.

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At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to have used the laser beam energy densities, permeabilization rates, foreign material, areas of the “defined area”, and laser beam path widths disclosed in Koller et al. when practicing the Kasuya invention. Furthermore, it would have been obvious to have expected the same probabilities of cell viability following permeabilization as determined by Koller et al.

One of ordinary skill in the art would have been motivated to do this because the parameters used by Koller et al. yielded high cell viability following irradiation with an energy beam. Furthermore, there would have been a reasonable expectation of success in obtaining the same range in cell viability by practicing the Kasuya by including features disclosed by Koller et al. Additionally, the selection of suitable laser beam energy densities, permeabilization rates, areas of the “defined area”, and laser beam path widths would have been a routine matter of optimizing result-effective parameters at the time of the invention. Thus, a holding of obviousness is clearly required.

Claims 1-3, 6-14, 17, 18, 21-34, and 45-56 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kasuya et al. and Koller et al. as applied to claims 1-3, 6-14, 17, 18, 23, 25-34, 45-48, 51, and 53-56 above, and further in view of Marchitto et al. (US 6,315,772).

As discussed above, Kasuya et al. and Koller et al. render claims 1-3, 6-14, 17, 18, 23, 25-34, 45-48, 51, and 53-56 obvious.

These references do not expressly disclose all the radiation durations recited in the claims, all membrane recovery times recited in the claims, radiation rates in terms of area per time, all pulse frequencies recited in the claims, and all energy sources recited in claim 25.

Marchitto et al. discloses a method wherein the surface of target skin tissue may be altered by irradiating the tissue with laser pulses, thus allowing for delivery of pharmaceuticals into the tissue cells (column 2, lines 42-52). Flashlamps (column 19, lines 24-25) and incandescent lights (column 19, lines 35-46), which are continuous lamps, may be used as sources of the electromagnetic energy for irradiating skin tissue. The laser pulse duration may be between 1 femtosecond to 1,000 microseconds (1 millisecond). Marchitto et al. discloses that the laser beam creates a "beam diameter at the skin in the range of 0.5 microns-5.0 cm" (column 7, lines 39-41). Using the formula of the area of a circle (πr^2), if the beam diameter is 5.0 cm, the area of the beam irradiated on the skin is about 20 cm². If the pulse duration is 1 millisecond, the rate of radiation would be 0.020 cm²/s, while the rate of radiation would be 2×10^{15} cm²/s if the pulse duration is 1 femtosecond. Thus, the electromagnetic radiation directed to an area of the tissue is at a rate of at least 0.0001, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 200, 300, or 400 cm²/s. Additionally, the pulse frequency may be in the range of 5×10^6 Hz to 3×10^7 Hz (column 12, lines 35-36), and may even be as high as 4 GHz (4×10^9 Hz) (column 12, lines 2-4). Therefore, the pulse frequency is at least 1, 10, 100, 10^3 , 10^4 , 10^5 , 10^6 , 10^7 Hz, 10^8 Hz, or 10^9 Hz.

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to have used the radiation durations, radiation rates in terms of area per time, pulse frequencies, and energy sources disclosed by Marchitto et al. when practicing the invention rendered obvious by Kasuya et al. and Koller et al. Furthermore, it would have been obvious to have optimized those parameter values and to have expected varying membrane recovery times.

One of ordinary skill in the art would have been motivated to do this since the Marchitto invention accomplished the same goal (delivery of compounds to cells through transient permeabilization of membranes) as was accomplished by the Kasuya and Koller inventions. There would have been a reasonable expectation of success in successfully permeabilizing membranes and delivering foreign material into the cells. Moreover, the selection of suitable radiation durations, radiation rates in terms of area per time, and pulse frequencies would have been a routine matter of optimizing result-effective parameters at the time of the invention. Finally, one would have expected a wide range of membrane recovery times following permeabilization since it would have been affected by equipment parameters (such as pulse duration). Thus, a holding of obviousness is clearly required.

Claims 1-14, 17, 18, 21-34, and 45-56 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kasuya et al., Koller et al., and Marchitto et al. as applied to claims 1-3, 6-14, 17, 18, 21-34, and 45-56 above, and further in view of Souhayer et al. (Anal. Chem., 2000, 72: 1342-1347).

As discussed above, Kasuya et al., Koller et al., and Marchitto et al. render claims 1-3, 6-14, 17, 18, 21-34, and 45-56 obvious.

These references do not expressly disclose effective distances recited in claims 4 and 5.

Souhayer et al. discloses the optoporation of cells, where cells are cultured in chambers comprising a cover slip, and the laser pulses are directed to the cover slips. Experiments were conducted for the delivery of a fluorophore into the cells. Souhayer et al. points out that “when the distance between the cells of interest and the laser beam is optimized, optoporation can be

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performed with good loading efficiency and high cellular survival rates” (page 1345, second column, second paragraph).

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to have varied the depth of the cell culture medium, which is the “effective distance”.

One of ordinary skill in the art would have been motivated to do this because Soughayer et al. indicates that it would have optimized loading efficiency and cellular survival rates. The selection of suitable “effective distances” would have been a routine matter of optimizing result-effective parameters at the time of the invention. Thus, a holding of obviousness is clearly required.

Claims 1-18, 21-34, and 45-56 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kasuya et al., Koller et al., Marchitto et al., and Soughayer et al. as applied to claims 1-14, 17, 18, 21-34, and 45-56 above, and further in view of Flock et al. (US 6,424,863).

As discussed above, Kasuya et al., Koller et al., Marchitto et al., and Soughayer et al. render claims 1-14, 17, 18, 21-34, and 45-56 obvious.

These references do not expressly disclose contacting the cells with an aqueous medium wherein certain molecules are released from the cells upon permeabilization.

Flock et al. teaches a method to enhance delivery of a pharmaceutical compound in a subject wherein the subject is irradiated with electromagnetic energy (claim 1). It is noted that “enhancement of drug delivery can take place with the use of osmotic or atmospheric pressure (applied, for example, in the form of a patch over the site of irradiation)” (column 4, lines 14-

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16). Furthermore, “a patch of distilled water in contact with the treated skin would enhance the diffusion of glucose out of the skin due to osmotic pressure”.

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to have practiced the Kasuya invention of transiently permeabilize cells in an aqueous medium lacking a substance, or containing the substance at a concentration lower than the concentration of the substance within the cells, such that the substance within the cells can exit the cell. Furthermore, the substances that may exit would be the same substances that can enter the cell as disclosed by Kasuya et al., Koller et al., Marchitto et al., and Soughayer et al.

One of ordinary skill in the art would have been motivated to do this since in order to control concentrations of therapeutic compounds in the cell, such as ions, organic molecules, inorganic molecules, colloidal particles, polysaccharides, peptides, proteins, nucleic acids, and modified nucleic acids. Thus, a holding of obviousness is required.

Applicant's arguments have been fully considered but they are not persuasive. The secondary cited references under §103 were discussed only to teach certain limitations in the dependent claims. As discussed above, Kasuya et al. teaches every limitation in the parent claims. Thus, the rejections under §103 must be maintained.

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire **THREE MONTHS** from the mailing date of this action. In the event a first reply is filed within **TWO MONTHS** of the mailing date of this final action and the advisory action is not mailed until after the end of the **THREE-MONTH** shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than **SIX MONTHS** from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Susan E. Fernandez whose telephone number is (571) 272-3444. The examiner can normally be reached on Mon-Fri 8:30 am - 5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mike Wityshyn can be reached on (571) 272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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